REMARKS

Claims 2-17 are in this application. Claim 1 has been cancelled, claims 9, 10, 14 and 16 have been amended and claims 2-8 are withdrawn.

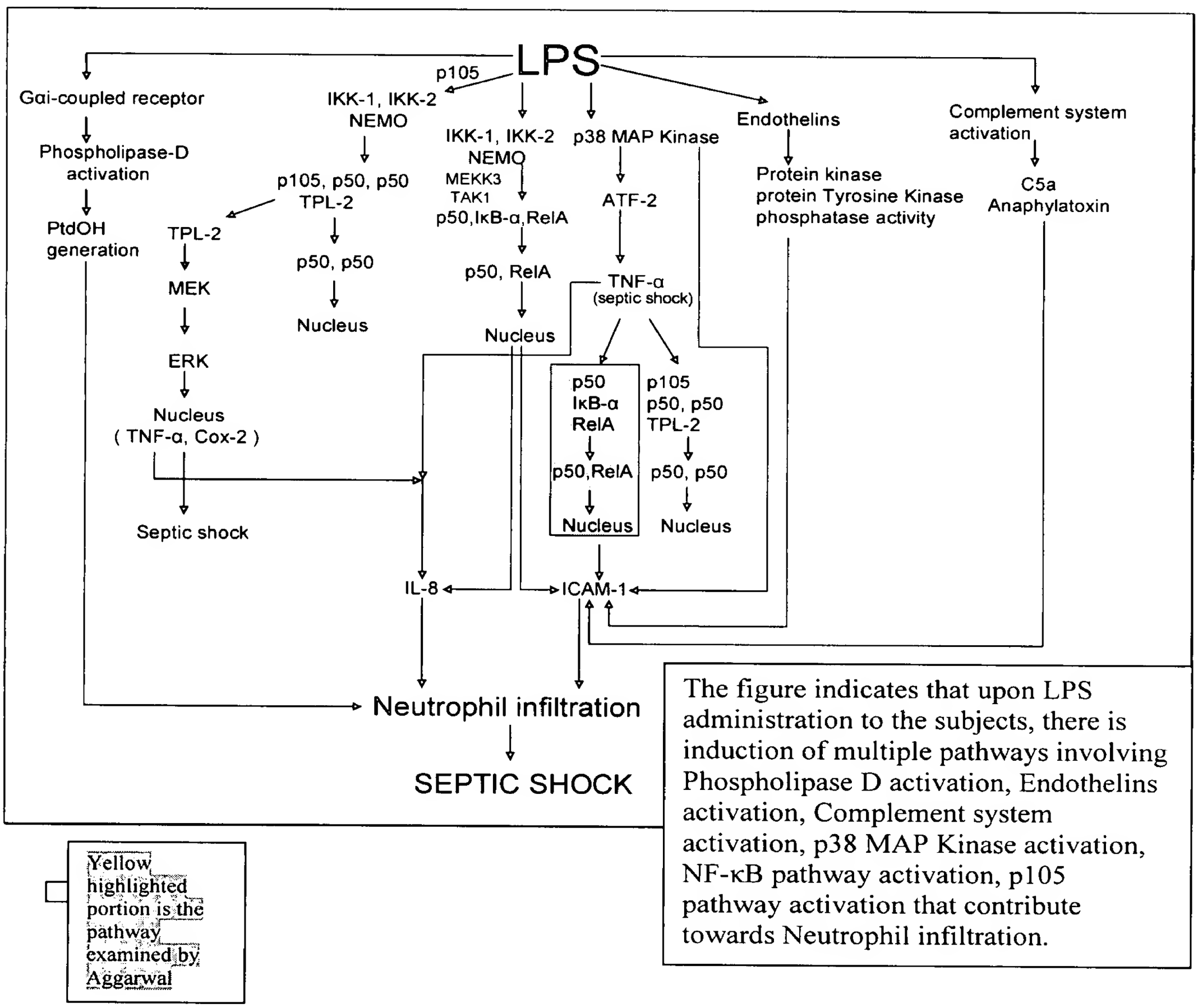
The specification has been amended to replace the description of Figures 2 (A), (B) and (C) with a brief description of Figures 2(A.), 2(a) 1, 2(a) 2 and 2(a) 3; Fig. 2(b); Fig. 2(b)1, 2(b)2 and 2(b)3; Fig. 2(c); Fig. 2(c)1; Fig. 2(c) 2; Fig. 2(c) 3; Fig. 2(c) 4 and Fig. 2(c) 5.

According to the Official Action, claims 14 and 15 are anticipated by WO97/09877 (Aggarwal); claims 9-12 and 16 are rejected under 35 USC 103(a) as being unpatentable over Aggarwal (WO9709877) in view of Ammon, et al. (U.S. 5,401,777); and Claims 13 and 17 are rejected under 35 USC 103(a) as being unpatentable over Aggarwal (WO 9709877) in view of Schneider (U.S. 6,013,273).

These rejections are respectfully traversed.

Septic shock condition arises upon severe infection with gram-negative bacteria. The lipopolysaccharide (LPS) that comprises the outer wall of the gram-negative bacteria activates various cells primarily macrophages, monocytes and other leukocytes. These activated cells release various mediators that lead to several pathophysiological reactions including fever, leukopenia, thrombocytopenia, intravascular coagulation, leukocyte infiltration and inflammation in various organs that may ultimately lead to death. Thus, there is a systemic inflammatory response to the invading pathogen. During septic shock **death is primarily caused due to liver damage which is caused by the excessive accumulation of neutrophils** in the liver tissue.

On induction of septic shock conditions by LPS treatment to mice, several inflammatory pathways are activated. The activated pathways are outlined in the figure shown below:



The figure indicates that upon LPS administration to the subjects, there is induction of multiple pathways involving Phospholipase D activation, Endothelins activation, Complement system activation, p38 MAP Kinase activation, NF-κB pathway activation, p105 pathway activation that contribute towards Neutrophil infiltration.

Yellow highlighted portion is the pathway examined by Aggarwal

Figure A: LPS induced pathways leading to Septic Shock

LPS injection induces septic shock conditions in a subject. Several inflammatory pathways are activated and different kinds of signaling mediators are released as shown in figure A above. The pathways include activation of Galphai coupled receptor, p105 mediated NEMO activation, release of IL-8, p38 MAP kinase, endothelin mediated kinase activation, complement system activation etc. Aggarwal teaches inhibition of TNF alpha induced NF kappa b activation by curcumin. The pathway of TNF alpha induced NF kappa b activation is **only** a part of the p38 MAP kinase pathway as shown in Figure A (highlighted part) above. Septic shock conditions only arise due to various pathways which are in operation such as Galphai coupled receptor, p105 mediated NEMO activation, release of IL-8, p38 MAP kinase, endothelin mediated kinase activation, complement system activation as shown in Figure A.

Aggarwal has induced NF kappa b activation by TNF alpha and extrapolated that it may alleviate septic shock conditions. Inhibition of NF kappa b activation by curcumin is not sufficient to prevent septic shock. Otherwise it would have been expected that strong inhibitors of NF kappa b such as aspirin, dichlorofenac and nimusilide etc. could have been useful to treat septic shock but in fact, these compounds can not be used for treatment of septic shock as is evidenced by the fact that septic shock would not be the problem that it is given that these compounds are relatively common. In fact inflammatory conditions arising due to uropathogenic *E. coli* gets further aggravated by inhibiting NFkappa b (Infect. Immun. 1001, Nov, 69 (11):6689-95).

Aggarwal has not examined LPS induced neutrophil infiltration and septic shock conditions. In contrast, the applicant used LPS and not TNF alpha to induce neutrophil infiltration leading to septic shock conditions which culminated in the death of the animal. In the instant invention this damage has been shown to be prevented in the curcumin pretreated animals which were injected with LPS. Similarly the animals exhibiting septic shock conditions could be revived by curcumin treatment. Similarly neutrophil infiltration was also observed to be reduced significantly by curcumin treatment in the experimental animals where

the recovery of cellular damage in liver could also be visualized (Figures 2 (a), 2 (a) 1-3, 2b, 2b (1-3) and 2c, 2c (1-5)).

Aggarwal states that "several additional, indirect lines of evidence suggest a role for ROI as a common and critical denominator including evidence that cellular levels of ROI increase in response to TNF, IL-1, PMA, LPS, UV light, and gamma irradiation. But among the various ROI administered to cells in culture, only hydrogen peroxide was found to be an effective activator of NF-.kappa.B "

This statement of Aggarwal clearly indicates that LPS could not activate NF kappa B in his cell culture experiments. Thus the anticipation and obviousness rejections are not relevant as the instant claims and Aggarwal's invention are originating from entirely different points. In applicant's experiments NF kappa b activation/inhibition could not have been the deciding factor at all and other pathways as shown in Figure A were operative leading to septic shock conditions. Hence it is requested that the anticipation and obviousness rejections in regard to Aggarwal be withdrawn.

Aggarwal also states that the present invention is also directed to a method of inhibiting the nuclear translocation of the p65 subunit of the NF-kB transcription factor in a cell in an animal in need of such treatment. Aggarwal states the preferably the human has a pathophysiological state selected from the group consisting of toxic/septic shock, graft vs. host reaction, radiation damage, atherosclerosis, AIDs, inflammation and cancer. There is no *in vivo* data in Aggarwal and there is nothing to support that administration of curcumin has any affect on any of these conditions. Aggarwal states that a method of inhibiting the phosphorylation and degradation of IkB protein in a cell or in an animal is relevant where the human has a pathophysiological state selected from the group consisting of toxic/septic shock, graft vs. host reaction, radiation damage, atherosclcerosis and cancer. Aggarwal is replete with speculative statements and does not provide any evidence that curcumin has any effect on these

conditions. In fact, on page 13 of the specification, Aggarwal speculates that intervention in NF-kB activation may be beneficial in suppressing toxic/septic shock, graft-vs-host reactions, acute inflammatory reactions, HIV replication, acute phase response and radiation damage. However, Aggarwal provides no evidence that intervention in NF-kB activation may be beneficial in suppressing toxic/septic shock, graft-vs-host reactions, acute inflammatory reactions, HIV replication, acute phase response and radiation damage and no evidence that curcumin activates NF-kb and that curcumin has any effect in vivo where numerous other systems are in play. Aggarwal did not disclose *that LPS could not activate NF kappa* B in his cell culture experiments. This clearly shows that these are two different types of responses with different origins. In addition, Aggarwal has not studied migration of neutrophils which is an important part of inflammatory response leading to septic shock condition.

Aggarwal et al.'s work is totally different from the claimed invention. No experiments with live animals were conducted. **LPS induced septic shock conditions** described above were not examined. Aggarwal, et al.'s treated human myeloid ML-1a cells with TNF-alpha and showed the activation of transcription factor NF-.kappa.B, which consists of p50 and p65 subunits. However, when they pretreated cells with curcumin followed by TNF-alpha stimulation, the activation of NF-.kappa.B was significantly inhibited.

It may be noted that TNF-α is only one of the mediators of septic shock. In the endotoxin shock model used by the applicant's, LPS is being administered to the animals to induce symptoms of septic shock. As explained above, septic shock is a pathological condition resulting from complex interactions of various mediators like cell adhesion molecules viz., ICAM-1, VCAM-1 and E-selection, P-selectin, L-selectin, proinflammatory cytokines and chemokines, like IL-1, TNF-a, nitric oxide, MIP-1a, CINC, CCR5, CSCR3 etc. whose expressions, in turn, may be up-regulated or down-regulated in septic shock condition based on the

intricate up or down regulation of various biochemical pathways and physiological mechanisms occuring at the *in vivo* level.

In the application in issue, it is demonstrated by in vivo experiments that curcumin inhibits neutrophil infiltration in mice and this prevents or protects from septic shock conditions.

Therefore, it is not justified to predict/or assume any agent for its therapeutic value just on the basis of *in vitro* experiments as enough contradictions and opposite effects have been observed in *in vivo* experiments. The reason behind this is clear. *In vitro* experiments of evaluating any compound show its effect on only one or few parameters. In the body thousands and more biological reactions are going on. An agent which affects only one of a few parameters *in vitro* may affect many other additional parameters also in the body which may or may not be favorable to alleviate the targeted disease. The agent will be anti-disease only when it really alleviates that particular targeted disease in the intact body.

As set out in applicant's specification, mice were injected **with bacterial lipopolysaccharide** to develop a model of septic shock as defined earlier. An excessive infiltration of neutrophils in the liver was observed in the dead animal which is one of the major reasons of septic shock induced death. Importantly, when mice were treated with curcumin, the lethality in mice injected with gram-negative bacterial lipopolysaccharide significantly reduced. Also the mice treated with curcumin are less lethargic, do not suffer from diarrhoea and their eyes are less watery; overall the severity of symptoms in the curcumin treated mice is much reduced compared to the mice injected with lipopolysaccharide alone. Also, treatment with curcumin prevents the infiltration of neutrophils into the liver tissue of the mice injected with gram-negative bacterial lipopolysaccharide. The neutrophils were found to be arrested in the sinusoids and do not extravasate

into the tissue. Thus, curcumin alleviates bacterial lipopolysaccharide induced septic conditions in mice.

As explained above, Aggarwal does not disclose the claimed invention. Aggarwal and Ammon in combination or Aggarwal and Schneider in combination do not make the claimed invention obvious. There is no disclosure or suggestion in these references that curcumin can be administered to treat toxic shock symptoms, that it has an affect on neutrophils and that it can be administered in an amount that is effective to prevent neutrophil infiltration from blood vessels to underlying tissues.

Therefore, it is submitted that all outstanding issues have been resolved and the present application is in condition for allowance.

Respectfully submitted,

JANET I. CORD
LADAS & PARRY LLP
26 WEST 61 STREET
NEW YORK, NEW YORK 10023
REG. NO. 33,778
(212) 708-1935